(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 479 916 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
20.11.1996 Bulletin 1996/47

(21) Application number: **90911356.5**

(22) Date of filing: **22.06.1990**

(51) Int Cl.[6]: **C07K 14/705**

(86) International application number:
PCT/US90/03564

(87) International publication number:
WO 90/15815 (27.12.1990 Gazette 1990/29)

(54) GAMMA RETINOIC ACID RECEPTOR

GAMMA-RETINOINSÄURE-REZEPTOR

RECEPTEUR D'ACIDE GAMMA RETINOIQUE

(84) Designated Contracting States:
AT BE CH DE ES FR GB IT LI LU NL SE

(30) Priority: **22.06.1989 US 370407**

(43) Date of publication of application:
15.04.1992 Bulletin 1992/16

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Printed by Jouve, 75001 PARIS (FR)

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- Nature, Volume 330, Issued 03 December 1987, (London England), PETKOVICH et al., "A Human Retinoic Acid Receptor which belongs to the family of Nuclear Receptors" pages 444-450, see entire document.
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Description

The present invention relates generally to ligand-responsive regulatory proteins and genes encoding them. More particularly, the present invention relates to a new retinoic acid receptor protein and the gene that encodes it, modification of the new retinoic acid receptor protein and gene by recombinant DNA and other genetic engineering techniques, plus uses of the new retinoic acid receptor protein and gene, both unmodified and modified.

BACKGROUND OF THE INVENTION

It is known that hormones like the glucocorticoid and thyroid hormones enter cells by facilitated diffusion. It is also known that hormones then bind to specific receptor proteins, thereby creating a hormone/receptor complex. The binding of hormone to the receptor initiates an allosteric alteration of the receptor protein. As a result of this alteration, it is believed that the hormone/receptor complex is capable of binding with high affinity to certain specific sites on the chromatin DNA. Such sites, which are referred to as hormone response elements or HRE's, modulate expression of nearby target gene promoters.

A major obstacle to further understanding of the specifics of gene regulation by exogenous inducers such as hormones has been the lack of availability of receptor proteins in sufficient quantity and purity to allow such proteins to be adequately analyzed and characterized. This same lack of availability has thwarted the use of receptors in diagnostic assays to determine the presence of exogenous inducers *(e.g.,* the hormones) in various body fluids and tissues, as well as their use as "prototypes" for engineering chimeric receptor protein analogs.

In an effort to overcome this lack of availability of receptor proteins, scientific investigators are working to discover the genes that encode such proteins. To date several such genes have been disclosed and characterized. The cloned genes include those encoding the following receptors: glucocorticoid, mineralocorticoid, progesterone, estrogen, the two steroid-related receptors (known in the art as ERR1 and ERR2), vitamin D_3, thyroid, v-*erb-A*, E75 *(Drosophilia)* and two retinoid receptor proteins, retinoic acid receptor alpha (RARα) and retinoic acid receptor beta (RARβ). *See* Giguere, *et al.,* (1987) regarding RARα, and Petkovich, *et al.,* (1987) and Brand, *et al.,* (1988) regarding RARβ.

In addition, Giguère et al. (Nature, vol. 337, 1989, pp. 566-569) have described the CDNA sequence encoding a new RAR.

This disclosure describes the isolation and characterization of a cDNA encoding a third functional retinoid receptor protein that is referred to herein as the gamma retinoic acid receptor (RARγ). Like RARs alpha and beta, the new gamma retinoic acid receptor has homology with the DNA-binding and ligand-binding domains of the steroid and thyroid hormone receptors.

The retinoic acid receptor genes belong to the superfamily of genes known as the steroid hormone receptor family. All genes in this family can be divided into discrete regions or domains that are sometimes referred to as regions A/B, C, D, E, and F. See Figure 2; also *see* Robertson, (1987) and Evans, (1988). The C region encodes the DNA-binding domain, the E region encodes the ligand-binding domain and the F region encodes the carboxy-terminus domain. The D region is believed to function as a "hinge". The function of the A/B (or N-terminus) region is not entirely clear; it may be involved with enhancement and repression of receptor transcription activity. *See* for example, Hollenberg, *et al.,* (1988) and Oro, *et al.,* (1988).

The present specification also discloses chimeric receptors made by "swapping" functional domains between the new gamma retinoic acid receptor and the glucocorticoid, the mineralocorticoid, the progesterone, the estrogen, the estrogen-related (ERR1 and ERR2), the vitamin D_3 receptor, the thyroid receptors, the v-*erb-A* receptor, the E75 *(Drosophilia)* receptor and the alpha and beta retinoic acid receptors. These chimeric receptors have hybrid functional characteristics based on the "origin" of the "parental" DNA-binding and ligand-binding domains incorporated within the chimeras. For example, if the DNA-binding domain in the chimeric receptor is the gamma retinoic acid receptor DNA-binding domain (*i.e.,* is obtained from wild-type gamma retinoic acid receptor or is a mutant that contains the functional elements of the gamma retinoic acid DNA-binding domain), then the chimera will have DNA-binding properties characteristic of the gamma retinoic acid receptor. The same is true of the ligand-binding domain.

DESCRIPTION OF THE DRAWINGS

The drawings comprise three figures of which:

FIGURE 1, which is in two parts, Fig. 1-1 and 1-2, is a drawing that shows the DNA nucleotide sequence and the primary protein sequence of hRARγ encoded by the *Eco*RI fragment harbored in pGEM-hRARγ.

FIGURE 2 is a drawing that shows the amino acid comparison among the three human RARs (alpha, beta and gamma).

FIGURE 3 (A and B) is composed of two blots. Fig. 3A shows induction of *CAT* activity and thus retinoic acid-

dependent transactivation by the protein encoded by the cDNA insert of pGEM-hRARγ. Fig. 3B shows that hRARγ recognizes ERE and TRE, but not GRE.

DEFINITIONS

In the present specification and claims, reference will be made to phrases and terms of art which are expressly defined for use herein as follows:

As used herein, the generic term "retinoids" means a group of compounds which includes retinoic acid, vitamin A (retinol) and a series of natural and synthetic derivatives that can exert profound effects on development and differentiation in a wide variety of systems.

As used herein, the human species is identified with a lower case "h".

As used herein, "steroid hormone superfamily of receptors" refers to the class of related receptors comprised of glucocorticoid, mineralocorticoid, progesterone, estrogen, estrogen-related (ERR1 and ERR2), vitamin D_3, thyroid, v-*erb-A*, E75 *(Drosophilia)* and the retinoic acid receptors. *See* Evans (1988) and the references cited therein.

As used herein, RAR means retinoic acid receptor. The acronym hRAR means human retinoic acid receptor. hRARα refers to human retinoic acid receptor alpha. *See* Giguere, *et al.*, (1987). hRARβ refers to human retinoic acid receptor beta. *See* Brand, *et al.*, (1988). hRARγ refers to human retinoic acid receptor gamma.

As used herein, GR means glucocorticoid receptor. hGR means human glucocorticoid receptor.

As used herein, MR means mineralocorticoid receptor. hMR means human mineralocorticoid receptor.

As used herein, T_3R means thyroid hormone receptor triiodthyronine. $T_3R\alpha$ and $T_3R\beta$ refer to the *alpha* and *beta* forms of the thyroid receptor.

As used herein, ER means estrogen receptor.

As used herein, ERR means estrogen-related receptor. The acronyms, hERR1 and hERR2 refer to human estrogen-related receptors 1 and 2. These receptors are more related to steroid receptors than to the thyroid receptors, yet they do not bind any of the major classes of known steroid hormones (Giguere, *et al.*, 1988).

As used herein, VDR means vitamin D_3 receptor.

As used herein, PR means progesterone receptor.

As used herein, *CAT* means chloramphenicol acetyltransferase.

As used herein, CV-1 means mouse kidney cells from the cell line referred to as "CV-1". CV-1 cells are receptor-deficient cells that are useful in functional ligand identification assays.

As used herein, hormone response elements or HRE's mean short *cis*-acting DNA sequences (about 20 bp in size) that are required for hormonal (or ligand) activation of transcription. The attachment of these elements to an otherwise hormone-nonresponsive promoter causes that promoter to become hormone responsive. These sequences function in a position- and orientation-independent fashion. Unlike other transcriptional regulators, the activity of the HRE's is dependent upon the presence or absence of ligand. See Evans (1988) and the references cited therein.

As used herein, synthetic HRE's refer to HRE's that have been synthesized *in vitro* using automated nucleotide synthesis machines. Since the HRE's are only about 20 bp in size, they are easily synthesized in this manner. If wild-type, engineered or synthetic HREs are linked to hormone-nonresponsive promoters, these promoters become hormone responsive. See Evans (1988) and the references cited therein.

As used herein, the acronym GRE means glucocorticoid response element and TRE means thyroid receptor response element. (TRE_P is a TRE that has been engineered to maximize the palindrominicity of this response element.) GRE's are hormone response elements that confer glucocorticoid responsiveness via interaction with the GR. *See* Payvar, *et al.*, *Cell* 35:381 (1983) and Schiedereit, *et al.*, *Nature* 304:749 (1983). GRE's can be used with any wild-type or chimeric receptor whose DNA-binding domain can functionally bind (*i.e.*, activate) with the GRE. For example, since GR, MR and PR receptors can all activate GRE's, a GRE can be used with any wild-type or chimeric receptor that has a GR, MR or PR-type DNA-binding domain. TRE's are similar to GRE's except that they confer thyroid hormone responsiveness via interaction with TR. TRE's can be used with any wild-type or chimeric receptor whose DNA-binding domain can functionally bind (*i.e.*, activate) with the TRE. Both thyroid and retinoic acid receptors can activate TRE's, so a TRE can be used with any receptor that has a TR or RAR-type DNA-binding domain.

As used herein, ligand means an inducer, such as a hormone or growth substance. Inside a cell, the ligand binds to a receptor protein, thereby creating a ligand/receptor complex, which in turn can bind to an appropriate hormone response element. Single ligands may have multiple receptors. For example, both the $T_3R\alpha$ and the $T_3R\beta$ bind thyroid hormone such as T_3.

As used herein, the phrase "DNA-binding domain" refers to that portion of the receptor protein (such as glucocorticoid, mineralocorticoid, progesterone, estrogen, estrogen-related receptors, vitamin D_3, thyroid, v-*erb-A*, E75 *(Drosophilia)* and the retinoic acid receptors) that binds to HRE sites on the chromatin DNA. The boundaries for these DNA-binding domains have been identified and characterized for the steroid hormone superfamily. *See* Evans (1988) and the references cited therein.

The DNA-binding domains of the steroid hormone superfamily of receptors consist of an amino acid segment varying between 66 to 68 amino acids in length. This segment contains 9 cysteine residues, one of which is the first amino acid of the segment. This first Cys residue begins a motif described as Cys-X_2-Cys-X_{13-15}-Cys-X_2-Cys, where X is any amino acid residue. The DNA-binding domain invariably ends with the amino acids Gly-Met.

For convenience in the cloning procedure, between 1 and 6 amino acids residues preceding and/or following the DNA-binding domain can be switched along with the DNA-binding domain.

As used herein, the phrase "ligand-binding domain region" refers to that portion of the receptor proteins that binds to ligands such as growth substances or hormones. These boundaries of the ligand-binding domains for the steroid receptor superfamily have been identified and characterized. See Evans (1988) and the references cited therein.

Common restriction endonuclease sites must be introduced into receptor cDNA clones to allow exchange of functional domains between receptors. In any of the various receptors in the steroid receptor superfamily of genes, the first common site can be introduced immediately preceding the DNA-binding domain, the second common site immediately following it. (For example, in any member of the steroid hormone superfamily, a unique *Not*I site can be introduced immediately preceding the region of the cDNA encoding the DNA-binding domain and a unique *Xho*I site can be introduced immediately following it. This divides the receptors into three functional regions or "cassettes"; (1) an N-terminus cassette, (2) a DNA-binding domain cassette, and (3) a ligand-binding domain cassette. The three regions or cassettes from any one receptor can be combined with cassettes from other receptors to create a variety of chimeric receptors.

As used herein, "mutant" DNA refers to DNA which has been genetically engineered to be different from the "wild-type" or unmodified sequence. Such genetic engineering can include the insertion of nucleotides into wild-type sequences, deletion of nucleotides from wild-type sequences, substitution of nucleotides in the wild-type sequences, or "swapping" of functional domains from one receptor to another. Receptors that have been engineered by "swapping" functional domains from one receptor to another are also referred to as chimeric or hybrid receptors. Chimeric receptors can be further engineered by insertion of nucleotides, deletion of nucleotides, substitution of nucleotides, *etc.*

Use of the term "substantial sequence homology" in the present specification and claims refers to DNA, RNA, or amino acid sequences that have slight and non-consequential sequence variations from the actual sequences disclosed and claimed herein and means that these sequences are within the scope of the appended claims. In this regard, the "slight and non-consequential" sequence variations mean that the homologous sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein.

As used herein, the term "recombinantly produced" means made using genetic engineering techniques, not merely purified from nature.

The amino acids which comprise the various amino acid sequences appearing herein may be identified according to the following three-letter or one-letter abbreviations:

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
L - Alanine	Ala	A
L - Arginine	Arg	R
L - Asparagine	Asn	N
L - Aspartic Acid	Asp	D
L - Cysteine	Cys	C
L - Glutamine	Gln	Q
L - Glutamic Acid	Glu	E
L - Glycine	Gly	G
L - Histidine	His	H
L - Isoleucine	Ile	I
L - Leucine	Leu	L
L - Lysine	Lys	K
L - Methionine	Met	M
L - Phenylalanine	Phe	F
L - Proline	Pro	P
L - Serine	Ser	S
L - Threonine	Thr	T
L - Tryptophan	Trp	W
L - Tyrosine	Tyr	Y
L - Valine	Val	V

The nucleotides which comprise the various nucleotide sequences appearing herein have their usual single-letter designations (A, G, T, C or U) used routinely in the art.

As used herein, bp means base pairs and kb means kilobase pairs.

DEPOSITS

Plasmid pCEM-hRARγ was deposited June 22, 1989 at the American Type Culture Collection, Rockville, Maryland, U.S.A. (ATCC) for patent purposes. It has been accorded ATCC No. 40623. The deposit of plasmid pGEM-hRARγ is under the terms of the Budapest Treaty on the International Recognition of Deposits of Microorganisms for Purposes of Patent Procedure and the Regulations promulgated under this Treaty. Samples of the plasmid are and will be available to industrial property offices and other persons legally entitled to receive it under the terms of said Treaty and Regulations and otherwise in compliance with the patent laws and regulations of the United States of America and all other nations or international organizations in which this application, or an application claiming priority of this application, is filed or in which any patent granted on any such application is granted.

DESCRIPTION OF THE INVENTION

In one aspect, the present invention comprises a double-stranded DNA segment wherein the plus or sense strand encodes the primary sequence of a protein that has ligand-binding and DNA-binding properties characteristic of a retinoid receptor protein referred to herein as human gamma retinoic acid receptor protein. According to this aspect of the invention, the double-stranded DNA segment is one which is capable of being expressed into human gamma retinoic acid receptor protein.

In another aspect, the invention comprises a single-stranded DNA, which is the sense strand of a double-stranded DNA coding for retinoic acid receptor gamma protein.

In another aspect, the invention comprises an mRNA made by transcription of the double-stranded DNA of the invention.

In another aspect, the invention comprises a plasmid, pGEM-hRARγ, which contains DNA encoding the human gamma retinoic acid receptor protein of the present invention (hRARγ). This plasmid has been deposited with the American Type Culture Collection for patent purposes; it has been accorded ATCC No. 40623.

In still another aspect, the invention comprises a recombinantly produced cell, preferably a recombinantly produced mammalian cell, engineered to contain DNA encoding retinoic acid receptor gamma protein. According to this aspect of the invention, the gamma retinoic acid encoding DNA is capable of being expressed in the recombinantly produced cell, thereby producing and/or increasing the amount of gamma retinoic acid receptor encoded by this DNA in the cell.

Further the invention comprises novel retinoic acid receptors made by expression of DNA encoding gamma retinoic acid receptor or translation of an mRNA transcribed from such gamma retinoic acid receptor encoding DNA. According to this aspect of the invention, the gamma retinoic acid receptors will be protein products of "unmodified" gamma retinoic acid receptor encoding DNA's and mRNA's, or will be modified or genetically engineered gamma retinoic acid receptor protein products which, as a result of engineered mutations in the receptor DNA sequences, will have one or more differences in amino acid sequence from the corresponding naturally occurring "wild-type" gamma retinoic acid receptor proteins. Preferably these gamma retinoic acid receptors, whether "unmodified" or "engineered", will have at least about 5% (over background) of the retinoic acid binding activity and/or at least about 5% (over background) of the DNA-binding or transcription-activating activity of the corresponding naturally occurring gamma retinoic acid receptor.

Further the invention comprises chimeric receptors made by exchanging the functional domains of the gamma retinoic acid receptor with functional domains of another type. The chimeric DNA's thus produced encode chimeric receptor proteins that have functional characteristics based on the "origin" of their respective DNA- and ligand-binding domains. The chimeric receptors of the invention include double-stranded DNA's that encode the chimeric receptors, as well as single-stranded DNA's which are the sense strands of the double-stranded DNA's, and mRNA's made by transcription of the double-stranded DNA's. The invention also comprises cells, both eukaryotic and prokaryotic, that are genetically engineered to contain chimeric receptor encoding DNA of the invention.

According to the preferred method for making the chimeric receptor genes and proteins of the present invention, to effect the chimeric DNA fusions, two restriction endonuclease sites are preferably introduced into each receptor DNA at comparable locations in or near the DNA-binding domains in order to divide the receptor DNA's into three functional domains or regions. (For example, a unique *Not*I site can be introduced immediately preceding the DNA-binding domain and a unique *Xho*I site can be introduced immediately following it.) This divides the receptors into three functional regions or "cassettes": (1) an N-terminus cassette, (2) a DNA-binding domain cassette, and (3) a ligand-binding domain cassette. The three regions or cassettes from the RARγ receptor can be combined with cassettes from other receptors from the steroid superfamily to create a variety of chimeric receptors.

The compositions, methods and assays of the invention, plus preferred methods for making and using them, are described more fully in the Examples that follow.

EXAMPLES

EXAMPLE 1

Isolation of the Gamma Retinoic Acid Receptor

An oligonucleotide from RARα was labeled and used to probe a human cDNA library constructed from human tumor liver cell mRNA. Nucleotide sequence analysis of one of the clones thus isolated revealed a long open reading frame of 454 amino acids beginning with a presumptive initiator methionine codon at position 200 as shown in Fig. 1.

EXAMPLE 2

RAR Amino Acid Sequence Comparison

The amino acid sequence of the newly discovered RARγ was compared with the amino acid sequences from RARα and hRARβ. The results of this comparison are shown in Fig. 2. As the drawing in the figure illustrates, remarkable identity in the amino acid sequence exists in the DNA-binding domains and in the ligand-binding domains.

EXAMPLE 3

Ligand Assay

To assay for the ligand for the putative new retinoic acid gamma receptor protein, the *Nco*I-*Eco*RI fragment of pGEM-hRARγ was recloned in the pRS eukaryotic expression vector giving pRshRARγ. The plasmid was introduced into monkey kidney CV-1 cells via calcium-phosphate transfection together with a reporter plasmid ΔMTV-TRE$_p$-*CAT*. As a control, pRS*erbA*$^{-1}$ (encodes no protein, stands as a negative control), pRShRARα, and pRShRARAβ were also examined. The transfected cells were incubated in the presence or absence of 100nM retinoic acid for 36 hours, and the induced *CAT* activities were analyzed by chromatography. The results indicate that a protein encoded by the *Nco*I-*Eco*RI insert transactivates through the ΔMTV-TRE$_p$ promoter in a retinoic acid dependent fashion, providing evidence that it is a functional new retinoic acid receptor. See Fig. 3A.

EXAMPLE 4

Response Element Specificity of hRARγ

To assay for the hormone response elements activated by the putative new gamma retinoic acid receptor protein, the *Nco*I-*Eco*RI fragment of pGEM-hRARγ was recloned in the pRS eukaryotic expression vector giving pRshRARγ. The plasmid was introduced into monkey kidney CV-1 cells via calcium-phosphate transfection together with one of the following reporter plasmids: ΔMTV-GRE-*CAT*, ΔMTV-ERE-*CAT*, or ΔMTV-TRE-*CAT*, with ΔMTV-*CAT* as the control. As above, the transfected cells were incubated in the presence or absence of 100nM retinoic acid for 36 hours, and the induced *CAT* activities were analyzed by chromatography. As Fig. 3B illustrates, hRARγ recognizes ERE and TRE, but not GRE, which is consistent with the other two known human retinoic acid receptors.

EXAMPLE 5

Gamma Retinoic Acid Receptor Data Summary

The data disclosed herein identify the protein product encoded by the cDNA insert in pGEM-hRARγ as human gamma retinoic acid receptor based on three criteria. First, the overall structural homology that the pGEM-hRARγ gene product has with hRARα and hRARβ suggests that it is a retinoic acid receptor. Second, the RARγ receptor protein acts as a transcriptional regulator of a TRE- or an ERE- inducible reporter gene in the presence of retinoic acid. Third, the hRARγ recognizes ERE and TRE, but not GRE.

REFERENCES

The present specification refers to the following publications, each of which is expressly incorporated by reference herein.

1. Brand, N., Petkovich, M., Krust, A., and Chambon, P., "Identification of a Second Human Retinoic Acid Receptor", *Nature* 332, 850-853 (1988).

2. Evans, R., "The Steroid and Thyroid Hormone Receptor Superfamily", *Science* 240, 889-895 (1988)

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9. Robertson, M., "Towards a Biochemistry of Morphogenesis", *Nature* 330, 420-421 (1987).

SPECIFICATION SUMMARY

From the foregoing description, one of ordinary skill in the art can understand that the present invention provides substantially pure DNA which encodes the retinoid receptor protein referred to as the gamma retinoic acid receptor protein. The invention also provides a plasmid containing the gamma retinoic acid receptor DNA. This plasmid, pGEM-hRARγ has been deposited with the American Type culture Collection for patent purposes.

The invention is also comprised of gamma retinoic acid receptor proteins, including modified functional forms thereof, expressed from the DNA (or mRNA) of the invention.

The present invention also includes chimeric hybrid receptors made by exchanging (1) the N-terminal domains, (2) the DNA-binding domains, and (3) the ligand-binding domains from hGR, hMR, ER, PR, hERR1, hERR2, T_3R_α, T_3R_β, VD_3R, v-*erb*-A, E75 and the alpha and beta RAR receptors with the domains of the new RARγ receptor. The chimeric receptors so constructed have DNA-binding domain and ligand-binding domain characteristics of the DNA-binding domain and ligand-binding domains of the respective "parental" receptors from which they originated.

The hRARγ DNA of the invention can be used to make the gamma retinoic acid receptor proteins, and functional modified forms thereof, in quantities that were not previously possible. The same is true of the chimeric receptors. With the quantities of gamma receptor protein available as a result of the present invention, the receptor proteins can be used to screen for gamma retinoic acid receptor-agonists or gamma retinoic acid receptor-antagonists. Availability of the gamma receptor proteins also means that they can be used in diagnostic assays to determine levels of retinoic acid present in various tissues and body fluids. Alternatively, the receptor proteins can be used to assay for levels of mRNA.

Claims

Claims for the following Contracting States : AT, BE,CH, LI, DE, FR, GB, IT, LU, NL, SE

1. Isolated DNA encoding protein which has ligand-binding and transcription-activating properties characteristic of gamma retinoic acid receptor protein and substantial amino acid homology with the sequence of amino acids shown in Figure 1.

2. The plasmid pGEM-hRARγ (ATCC No. 40623).

3. Isolated DNA encoding human gamma retinoic acid receptor.

4. Isolated DNA having substantial sequence homology with any one of the DNA's claimed in any one of Claims 1-3.

5. A genetically engineered mutant of any of the isolated DNA's claimed in any one of Claims 1-4 wherein said mutant DNA encodes protein which has ligand-binding and transcription-activating properties characteristic of gamma

retinoic acid receptor protein.

6. Recombinantly produced protein wherein the amino acid sequence comprising the DNA binding domain has at least about 98% homology with the amino acid sequence shown in Figure 1.

7. Protein encoded by any of the isolated DNA's claimed in any one of Claims 1-5

8. Recombinantly produced cells engineered to express any one of the isolated DNA's claimed in any of Claims 1-5.

9. Chimeric receptors having at least two functional domains wherein at least one of the domains is selected from the group consisting of the RARγ N-terminus domain, the RARγ DNA-binding domain, and the RARγ ligand-binding domain, and at least one of the remaining domains is selected from the group consisting of N-terminus, DNA-binding and ligand-binding domains from the glucocorticoid receptor, the mineralocorticoid receptor, the progesterone receptor, the estrogen receptor, the steroid-related receptors (ERR1 and ERR2), the vitamin D_3 receptor, the thyroid receptors, the v-*erb-A* receptor, the E75 *(Drosophilia)* receptor, and the retinoic acid receptors alpha and beta.

Claims for the following Contracting State : ES

1. A method for the production of a DNA encoding protein which has ligand-binding and transcription-activating properties characteristic of gamma retinoic acid receptor protein and substantial amino acid homology with the sequence of amino accids shown in Figure 1, wherein said DNA is isolated.

2. A method for the production of the plasmid pGEM-hRARγ (ATCC No. 40623), wherein said plasmid is isolated.

3. A method for the production of a DNA encoding human gamma retinoic acid receptor, wherein said DNA is isolated.

4. A method for the production of a DNA having substantial sequence homology with any one of the DNAs claimed in any one of Claims 1-3.

5. A method for the production of a mutant of any of the isolated DNAs claimed in any one of Claims 1-4 wherein said mutant DNA is genetically engineered and encodes protein which has ligand-binding and transcription-activating properties characteristic of gamma retinoic acid receptor protein.

6. A method for the production of a protein wherein the amino acid sequence comprising the DNA binding domain has at least about 98 % homology with the amino acid sequence shown in Figure 1, wherein said protein is recombinantly produced.

7. A method for the production of a protein encoded by any of the isolated DNAs claimed in any one of Claims 1-5.

8. A method for the production of cells engineered to express any one of the isolated DNAs claimed in any of Claims 1-5, wherein said cells are recombinantly produced.

9. A method for the production of chimeric receptors having at least two functional domains wherein at least one of the domains is selected from the group consisting of the RARγ N-terminus domain, the RARγ DNA-binding domain, and the RARγ ligand-binding domain, and at least one of the remaining domains is selected from the group consisting of N-terminus, DNA-binding and ligand-binding domains from the glucocorticoid receptor, the mineralocorticoid receptor, the progesterone receptor, the estrogen receptor, the steroid-related receptors (ERR1 and ERR2), the vitamin D_3 receptor, the thyroid receptors, the v-*erb*-A receptor, the E75 *(Drosophilia)* receptor, and the retinoic acid receptors alpha and beta, wherein said receptors are recombinantly produced.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE,CH, LI, DE, FR, GB, IT, LU, NL, SE

1. Isolierte DNA, welche ein Protein kodiert, das Ligand-bindende und Transkriptions-aktivierende Eigenschaften aufweist, die charakteristisch für das Gamma-Retinoinsäure-Rezeptorprotein sind, und eine wesentliche Aminosäurehomologie mit der in Fig. 1 gezeigten Aminosäuresequenz zeigt.

2. Plasmid pGEM-hRARγ (ATCC Nr. 40623).

3. Isolierte DNA, die den humanen gamma-Retinoinsäurerezeptor kodiert.

4. Isolierte DNA mit einer wesentlichen Sequenzhomologie mit einer der DNA's, nach einem der Ansprüche 1 bis 3

5. Gentechnisch veränderte Mutante von einer der isolierten DNA's nach einem der Ansprüche 1 bis 4, worin die mutierte DNA ein Protein kodiert, das Ligand-bindende und Transkriptions-aktivierende Eigenschaften hat, die charakteristisch für das gamma-Retinoinsäure-Rezeptorprotein sind.

6. Rekombinant produziertes Protein, worin die Aminosäuresequenz, welche die DNA-Bindungsdomäne umfaßt, mindestens eine Homologie von ca. 98 % mit der Aminosäuresequenz, wie in Fig. 1 gezeigt, hat.

7. Protein, kodiert durch eine der isolierten DNA's nach einem der Ansprüche 1 bis 5.

8. Rekombinant produzierte Zellen, die gentechnisch und zur Expression von einer der isolierten DNA's nach einem der Ansprüche 1 bis 5 verändert wurden.

9. Chimäre Rezeptoren mit mindestens zwei funktionellen Domänen, worin mindestens eine der Domäne ausgewählt ist aus Rarγ-N-terminaler Domäne, RARγ-DNA-Bindungsdomäne und der RARγ-Ligand-Bindungsdomäne, und mindestens eine der übrigen Domänen ausgewählt ist aus der N-terminalen DNA-Bindungs- und Ligand-Bindungsdomänen aus dem Glucocorticoidrezeptor, dem Mineralcorticoidrezeptor, dem Progesteronrezeptor, dem Östrogenrezeptor, den Steroid-verwandten Rezeptoren (ERR1 und ERR2), dem Vitamin-D_3-Rezeptor, den Thyroidrezeptoren, dem v-erb-A-Rezeptor, dem E75 (Drosophilia)-Rezeptor und den Retinoinsäurerezeptoren alpha und beta.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung einer Protein-kodierenden DNA, welche Ligand-bindende und Transkriptions-aktivierende Eigenschaften hat, die charakteristisch für das Gamma-Retinoinsäure-Rezeptorprotein sind, und die eine wesentliche Aminosäurehomologie mit der Sequenz der in Fig. 1 gezeigten Aminosäuresequenzen, wobei die DNA isoliert wird.

2. Verfahren zur Herstellung des Plasmids pGEM-hRARγ (ATCC Nr. 40623), worin das Plasmid isoliert wird.

3. Verfahren zur Herstellung von humaner gamma-Retinoinsäurerezeptor-kodierender DNA, worin die DNA isoliert wird.

4. Verfahren zur Herstellung einer DNA mit einer wesentlichen Sequenzhomologie mit einer der DNA's, gemäß einem der Ansprüche 1 bis 3.

5. Verfahren zur Herstellung einer Mutanten von einer der isolierten DNA's nach einem der Ansprüche 1 bis 4, worin die mutierte DNA gentechnisch verändert wurde und ein Protein kodiert, das Ligand-bindende und Transkriptions-aktivierende Eigenschaften hat, welche charakteristisch für das gamma-Retinoinsäure-Rezeptorprotein sind.

6. Verfahren zur Herstellung eines Proteins, worin die Aminosäuresequenz, umfassend die DNA-Bindungsdomäne, mindestens eine ca. 98%ige Homologie mit der in Fig. 1 gezeigten Aminosäuresequenz hat, worin das Protein rekombinant produziert wurde.

7. Verfahren zur Herstellung eines Proteins, kodiert durch eine der isolierten DNA's nach einem der Ansprüche 1 bis 5.

8. Verfahren zur Herstellung von gentechnisch veränderten Zellen unter Expression einer der isolierten DNA's nach einem der Ansprüche 1 bis 5, worin die Zellen rekombinant produziert werden.

9. Verfahren zur Herstellung chimärer Rezeptoren mit mindestens zwei funktionellen Domänen, worin mindestens eine der Domäne ausgewählt ist aus RARγ-N-terminalen Domäne, RARγ-DNA-Bindungsdomäne und der RARγ-Ligand-Bindungsdomäne, und mindestens eine der übrigen Domänen ausgewählt ist aus der N-terminalen DNA-Bindungs- und Ligand-Bindungsdomänen aus dem Glucocorticoidrezeptor, dem Mineralcorticoidrezeptor, dem Progesteronrezeptor, dem Östrogenrezeptor, de Steroid-verwandten Rezeptoren (ERR1 und ERR2), dem Vitamin-D_3-Rezeptor, den Thyroidrezeptoren, dem v-erb-A-Rezeptor, dem E75 (Drosophilia)-Rezeptor und den Retinoinsäurerezeptoren alpha und beta, worin die Rezeptoren rekombinant hergestellt wurden.

Revendications

Revendications pour les Etats contractants suivants : AT, BE,CH, LI, DE, FR, GB, IT, LU, NL, SE

1. ADN isolé codant une protéine qui possède des propriétés de liaison au ligand et d'activation de la transcription caractéristiques d'une protéine récepteur gamma d'acide rétinoïque et dont la séquence d'acides aminés présente un degré important d'homologie avec la séquence d'acides aminés représentée sur la figure 1.

2. Plasmide pGEM-hRARγ (ATCC n° 40623).

3. ADN isolé codant un récepteur gamma humain d'acide rétinoïque.

4. ADN isolé dont la séquence présente un degré important d'homologie avec celle de l'un des ADN conformes à l'une des revendications 1 à 3.

5. Mutant, obtenu par génie génétique, de l'un des ADN isolés conformes à l'une des revendications 1 à 4, cet ADN mutant codant une protéine qui possède des propriétés de liaison au ligand et d'activation de la transcription caractéristiques d'une protéine récepteur gamma d'acide rétinoïque.

6. Protéine produite par recombinaison, dont la séquence d'acides aminés comportant le domaine de liaison à l'ADN présente au moins environ 98 % d'homologie avec la séquence d'acides aminés représentée sur la figure 1.

7. Protéine codée par l'un des ADN isolés conformes à l'une des revendications 1 à 5.

8. Cellules produites par recombinaison génétique et manipulées pour qu'elles expriment l'un des ADN isolés conformes à l'une des revendications 1 à 5.

9. Récepteurs chimériques comportant au moins deux domaines fonctionnels, dans lesquels au moins l'un de ces domaines est choisi dans l'ensemble constitué par le domaine N-terminal de RARγ, le domaine de liaison à l'ADN de RARγ et le domaine de liaison au ligand de RARγ, et au moins l'un des autres domaines est choisi dans l'ensemble constitué par les domaines N-terminaux, les domaines de liaison à l'ADN et les domaines de liaison au ligand des récepteurs de glucocorticoïdes, des récepteurs de minéralocorticoïdes, des récepteurs de la progestérone, des récepteurs d'oestrogènes, des récepteurs ERR1 et ERR2 d'hormones apparentées aux stéroïdes, des récepteurs de la vitamine D_3, des récepteurs d'hormones thyroïdiennes, du récepteur E75 *(Drosophilia)*, du récepteur v-*erb-A*, et des récepteurs alpha et bêta d'acide rétinoïque.

Revendications pour l'Etat contractant suivant : ES

1. Procédé de production d'un ADN codant une protéine qui possède des propriétés de liaison au ligand et d'activation de la transcription caractéristiques d'une protéine récepteur gamma d'acide rétinoïque et dont la séquence d'acides aminés présente un degré important d'homologie avec la séquence d'acides aminés représentée sur la figure 1, dans lequel procédé ledit ADN est isolé

2. Procédé de production du plasmide pGEM-hRARγ (ATCC n° 40623), dans lequel ledit plasmide est isolé.

3. Procedé de production d'un ADN codant un récepteur gamma humain d'acide rétinoïque, dans lequel procédé ledit ADN est isolé.

4. Procedé de production d'un ADN dont la séquence présente un degré important d'homologie avec celle de l'un des ADN produits selon l'une des revendications 1 à 3.

5. Procedé de production d'un mutant de l'un des ADN isolés produits selon l'une des revendications 1 à 4, dans lequel ledit ADN mutant est obtenu par génie génétique et code une protéine qui possède des propriétés de liaison au ligand et d'activation de la transcription caractéristiques d'une protéine récepteur gamma d'acide rétinoïque.

6. Procedé de production d'une protéine dont la séquence d'acides aminés comportant le domaine de liaison à l'ADN présente au moins environ 98 % d'homologie avec la séquence d'acides aminés représentée sur la figure 1, dans lequel procédé ladite protéine est produite par recombinaison.

7. Procedé de production d'une protéine codée par l'un des ADN isolés produits selon l'une des revendications 1 à 5.

8. Procedé de production de cellules génétiquement manipulées pour qu'elles expriment l'un des ADN isolés produits selon l'une des revendications 1 à 5, dans lequel lesdites cellules sont produites par recombinaison.

9. Procedé de production de récepteurs chimériques comportant au moins deux domaines fonctionnels, dans lesquels au moins l'un de ces domaines est choisi dans l'ensemble constitué par le domaine N-terminal de RARγ, le domaine de liaison à l'ADN de RARγ et le domaine de liaison au ligand de RARγ, et au moins l'un des autres domaines est choisi dans l'ensemble constitué par les domaines N-terminaux, les domaines de liaison à l'ADN et les domaines de liaison au ligand des récepteurs de glucocorticoïdes, des récepteurs de minéralocorticoïdes, des récepteurs de la progestérone, des récepteurs d'oestrogènes, des récepteurs ERR1 et ERR2 d'hormones apparentées aux stéroïdes, des récepteurs de la vitamine D_3, des récepteurs d'hormones thyroïdiennes, du récepteur E75 *(Drosophila)*, du récepteur v-*erb-A*, et des récepteurs alpha et bêta d'acide rétinoïque, dans lequel procédé lesdits récepteurs sont produits par recombinaison.

FIG. 1 – 1

```
   1                                                GAATTCGGCTCAACCTGACCCAGTATGTAGAAGCCAGTCTC
  43 TGCAGGCGGCCAGCGGGACTTTTGGAGGCCCAGTGGGCAGGCCAGGCAGGGCGGGTACGGAGCCTCCCAGGCTGGGGCA
 121 GTGGGCATGGGCAGGGGCTGTGGCTGAAGACCTCGCCCGCCCACTGCAGACCCCAGGGGACTCTCACACCGCAGCTGCC

 200 ATG GCC ACC AAT AAG GAG CGA CTC TTT GCG GCT GGT GCC CTG GGG CCT GGA TCT GGC TAC
   1 Met Ala Thr Asn Lys Glu Arg Leu Phe Ala Ala Gly Ala Leu Gly Pro Gly Ser Gly Tyr

 260 CCA GGG GCA GGT TTC CCC TTC GCC TTC CCA GGG GCA CTC AGG GGG TCT CCG CCT TTC GAG
  21 Pro Gly Ala Gly Phe Pro Phe Ala Phe Pro Gly Ala Leu Arg Gly Ser Pro Pro Phe Glu

 320 ATG CTG AGC CCT AGC TTC CGG GGC CTG GGC CAG CCT GAC CTC CCC AAG GAG ATG GCC TCT
  41 Met Leu Ser Pro Ser Phe Arg Gly Leu Gly Gln Pro Asp Leu Pro Lys Glu Met Ala Ser

 380 CTG TCG GTG GAG ACA CAG AGC ACC AGC TCA GAG GAG ATG GTG CCC AGC TCG CCC TCG CCC
  61 Leu Ser Val Glu Thr Gln Ser Thr Ser Ser Glu Glu Met Val Pro Ser Ser Pro Ser Pro

 440 CCT CCG CCT CCT CGG GTC TAC AAG CCA TGC TTC GTG TGC AAT GAC AAG TCC TCT GGC TAC
  81 Pro Pro Pro Pro Arg Val Tyr Lys Pro Cys Phe Val Cys Asn Asp Lys Ser Ser Gly Tyr

 500 CAC TAT GGG GTC AGC TCT TGT GAA GGC TGC AAG GGC TTC TTT CGC CGA AGC ATC CAG AAG
 101 His Tyr Gly Val Ser Ser Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Ser Ile Gln Lys

 560 AAC ATG GTG TAC ACG TGT CAC CGC GAC AAA AAC TGT ATC ATC AAC AAG GTG ACC AGG AAT
 121 Asn Met Val Tyr Thr Cys His Arg Asp Lys Asn Cys Ile Ile Asn Lys Val Thr Arg Asn

 620 CGC TGC CAG TAC TGC CGG CTA CAG AAG TGC TTC GAA GTG GGC ATG TCC AAG GAA GCT GTG
 141 Arg Cys Gln Tyr Cys Arg Leu Gln Lys Cys Phe Glu Val Gly Met Ser Lys Glu Ala Val

 680 CGA AAT GAC CGG AAC AAG AAG AAG AAA GAG GTG AAG GAA GAA GGG TCA CCT GAC AGC TAT
 161 Arg Asn Asp Arg Asn Lys Lys Lys Lys Glu Val Lys Glu Glu Gly Ser Pro Asp Ser Tyr

 740 GAG CTG AGC CCT CAG TTA GAA GAG CTC ATC ACC AAG GTC AGC AAA GCC CAT CAG GAG ACT
 181 Glu Leu Ser Pro Gln Leu Glu Glu Leu Ile Thr Lys Val Ser Lys Ala His Gln Glu Thr
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```
800 TTC CCC TCG CTC TGC CAG CTG GGC AAG TAT ACC ACG AAC TCC AGT GCA GAC CAC CGC GTG
201 Phe Pro Ser Leu Cys Gln Leu Gly Lys Tyr Thr Thr Asn Ser Ser Ala Asp His Arg Val

860 CAG CTG GAT CTG GGG CTG TGG GAC AAG TTC AGT GAG CTG GCT ACC AAG TGC ATC ATC AAG
221 Gln Leu Asp Leu Gly Leu Trp Asp Lys Phe Ser Glu Leu Ala Thr Lys Cys Ile Ile Lys

920 ATC GTG GAG TTT GCC AAG CGG TTG CCT GGC TTT ACA GGG CTC AGC ATT GCT GAC CAG ATC
241 Ile Val Glu Phe Ala Lys Arg Leu Pro Gly Phe Thr Gly Leu Ser Ile Ala Asp Gln Ile

980 ACT CTG CTC AAA GCT GCC TGC CTA GAT ATC CTG ATG CTG CGT ATC TGC ACA AGG TAC ACC
261 Thr Leu Leu Lys Ala Ala Cys Leu Asp Ile Leu Met Leu Arg Ile Cys Thr Arg Tyr Thr

1040 CCA GAG CAG GAC ACC ATG ACC TTC TCC GAC GGG CTG ACC CTG AAC CGG ACC CAG ATG CAC
281 Pro Glu Gln Asp Thr Met Thr Phe Ser Asp Gly Leu Thr Leu Asn Arg Thr Gln Met His

1100 AAT GCC GGC TTC GGG CCC CTC ACA GAC CTT GTC TTT GCC TTT GCT GGG CAG CTC CTG CCC
301 Asn Ala Gly Phe Gly Pro Leu Thr Asp Leu Val Phe Ala Phe Ala Gly Gln Leu Leu Pro

1160 CTG GAG ATG GAT GAC ACC GAG ACA GGG CTG CTC AGC GCC ATC TGC CTC ATC TGC GGA GAC
321 Leu Glu Met Asp Asp Thr Glu Thr Gly Leu Leu Ser Ala Ile Cys Leu Ile Cys Gly Asp

1220 CGC ATG GAC CTG GAG GAG CCC GAA AAA GTG GAC AAG CTG CAG GAG CCA CTG CTG GAA GCC
341 Arg Met Asp Leu Glu Glu Pro Glu Lys Val Asp Lys Leu Gln Glu Pro Leu Leu Glu Ala

1280 CTG AGG CTG TAC GCC CGG CGC CGG CGG CCC AGC CAG CCC TAC ATG TTC CCA AGG ATG CTA
361 Leu Arg Leu Tyr Ala Arg Arg Arg Arg Pro Ser Gln Pro Tyr Met Phe Pro Arg Met Leu

1340 ATG AAA ATC ACC GAC CTC CGG GGC ATC AGC ACT AAG GGA GCT GAA AGG GCC ATT ACT CTG
381 Met Lys Ile Thr Asp Leu Arg Gly Ile Ser Thr Lys Gly Ala Glu Arg Ala Ile Thr Leu

1400 AAG ATG GAG ATT CCA GGC CCG ATG CCT CCC TTA ATC CGA GAG ATG CTG GAG AAC CCT GAA
401 Lys Met Glu Ile Pro Gly Pro Met Pro Pro Leu Ile Arg Glu Met Leu Glu Asn pro Glu

1460 ATG TTT GAG GAT GAC TCC TCG CAG CCT GGT CCC CAC CCC AAT GCC TCT AGC GAG GAT GAG
421 Met Phe Glu Asp Asp Ser Ser Gln Pro Gly Pro His Pro Asn Ala Ser Ser Glu Asp Glu

1520 GTT CCT GGG GGC CAG GGC AAA GGG GGC CTG AAG TCC CCA GCC TGA CCA GGC CGA ATT
441 Val Pro Gly Gly Gln Gly Lys Gly Gly Leu Lys Ser Pro Ala End
```

FIG. 1—2

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HUMAN RETINOIC ACID RECEPTORS

[amino terminal] A/B REGION FIG. 2

```
γ      MATNKERLFAAGALGPGSGYPGAGFPFAFPGALRGSPPFEMLSPSFRGLGQPDLPKEMAS
α        MASNSSSCPTPG.GHLNGYPVPPYAF.FPPML.GLSPPGALTTLQHQLPVSGYSTPSP
β              MFDCMDVL.VS..QILD.YTASPSSCMLQEKA.KAC.S..T.TEWQHRHTA

γ      LSVETQSTSSEEMVPSSPSPPPPPRVYKP 89
α      ATI....S....I...P.....L..I... 87
β      Q.I.........L...P...L........ 80
```

[DNA binding] C REGION

```
γ  90 CFVCNDKSSGYHYGVSSCEGCKGFFRRSIQKNMVYTCHRDKNCIINKVTRNRCQYCRLQK
α  88 ....Q...........A.....................................
β  81 ....Q...........A.............I.........V..............

γ      CFEVGM 155
α      ...... 153 (97%)
β      ...... 146 (94%)
```

[hinge] D REGION

```
γ 156 SKEAVRNDRNKKKKEVKEEGSPDSYELSPQLEELITKVSKAHQETF 201
α 154 ...S...........PKPECSE..T.T.EVG...E..R....... 199
β 147 ...S...........TSKQECTE...MTAE.DD.TE.IR....... 192
```

[ligand binding] E REGION

```
γ 202 PSLCQLGKYTTNSSADHRVQLDLGLWDKFSELATKCIIKIVEFAKRLPGFTGLSIADQIT
α 200 .A.........N.SEQ..S..ID........S......T.....Q.....T.T......
β 193 ...................R.................................T......

γ      LLKAACLDILMLRICTRYTPEQDTMTFSDGLTLNRTQMHNAGFGPLTDLVFAFAGQLLPL
α      .........I.....................................................N.....
β      .........I.....................................................T..N.....

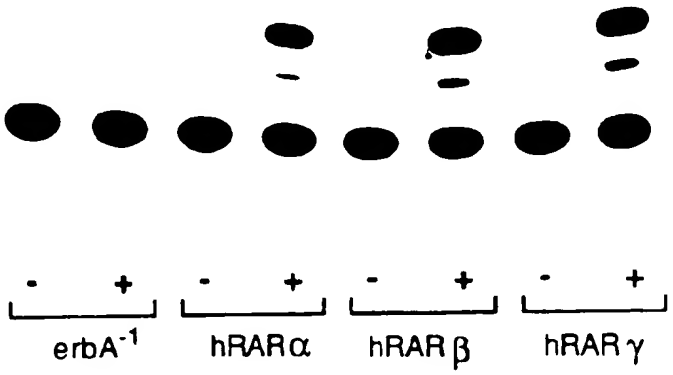
γ      EMDDTETGLLSAICLICGDRMDLEEPEKVDKLQEPLLEALRLYARRRRPSQPYMFPRMLM
α      ....A..............Q...Q.DR..M.........KV.V.K....R.H...K...
β      ...................Q.....T.............KI.I.K....K.H...KI..

γ      KITDLRGISTKGAERAITLKMEIPGPMPPLIREMLENPEM 421
α      ......S..A.....V.........S.....Q.....S.G 419 (84%)
β      ......S..A.....V.........S.....Q..M..S.G 412 (90%)
```

[carboxyl terminal] F REGION

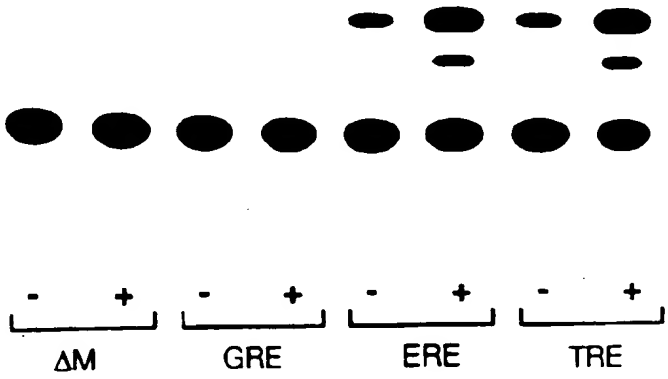
```
γ 422 FEDDSSQPGPHPNASSEDEVPGGQGKGGLKSPA* 454
α 420 LDTL.G...GGGRDGGGLAP.P.SCSPS.SPSSNRSSPATHSP* 462
β 413 H.PLTPSSSGNTAEH.PSIS.SSVENS.VSQSPLVQ* 448
```

γ = GAMMA RETINOIC ACID RECEPTOR
α = ALPHA RETINOIC ACID RECEPTOR
β = BETA RETINOIC ACID RECEPTOR



Reporter: ΔM-TREp-CAT

FIG. 3A



Receptor: pRShRARγ

FIG. 3B